Rejection under 35 U.S.C. § 102

Claims 24-26 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Lim *et al.*, "Detection of Group D Samonallae in Blood Culter Broth and of Soluble Antigen by Tube Agglutination Using an O-9 Monoclonal Antibody Latex Conjugate," J. OF CLIN. MICROBIOL., (July 1987) 25(7): 1165-1168 ("Lim").

Claim 24 is directed to an agglutination reagent for rapid and early detection of typhoid. The reagent comprises 1% carboxylated latex particles coated with antibody specific to *Salmonella typhi*, suspended in storage buffer.

On page 5, the Office Action contends that Lim's abstract teaches latex particles coated with a monoclonal antibody specific for *Salmonella* typhi. However, Lim does not teach ***carboxylated*** latex particles. To address this issue, the Office Action states that "the carboxylated latex beads are commercially available from Sigma (page 1165, col. 2)." It is believed that the Office Action is relying on this statement from Lim: "[a] 1% suspension of latex particles (diameter 0.797 μm; Sigma Chemical Co., Ltd. Poole, United Kingdom) was sensitized in 0.1 M glycine–0.9% sodium chloride buffer (pH 8.2) with an equal colume of *Salmonella* O-9 monoclonal immunoglobulin M (IgM) antibodies (7) by a previously described method (5)" (Lim at page 1165, col. 2). This portion does not teach the use of carboxylated latex particles. Instead, it teaches the use of non-carboxylated latex beads. In contrast, the invention recited in claim 24 is directed to carbolxylated latex particles.

The monoclonal antibodies taught by Lim are made against 0-9 antigen of *Salmonella* (Lim at abstract). Thus, the antibodies are specific to *Salmonella* typhi and *Salmonella* panama (Lim at page 1165, col. 1). In contrast, claim 24 recites that the antibody is specific to *Salmonella* typhi.

Thus, Lim does not teach each and every limitation recited in claims 24-26. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

Rejection under 35 U.S.C. § 103

Claims 23-27 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Nilsson *et al.* "Microparticles for selective protein determination in capillary electrophoresis," ELECTROPHORESIS, (2001) 22: 2384-2390 ("Nilsson") and Salzman *et al.* (WO 01/040280) ("Salzman"), in view of Sukosol *et al.*, "Fusion protein of Solmonella typhi flagellin as antigen for diagnosis of typhoid fever," ASIAN PACIFIC J. OF ALLERGY AND IMMUN., (1994) 12:21-25 ("Sukosol").

In part, claim 23 recites a process for the preparation of an agglutination reagent for rapid and early detection of typhoid comprising preparing antibody specific to *Salmonella* typhi, preparing latex particles suspension, and coating of the said latex particles with the said antibody. The antibody specific to *Salmonella* typhi is prepared in part by cloning a Flagellin gene sequence specific to *Salmonella* typhi, expressing the Flagellin gene sequence. The latex particle suspension is prepared in part by mixing 1% carboxylated latex particles of size 0.88 to 0.90 μm and 40 mM 2-N morpholinoethane sulphonic acid (MES) buffer of pH 5.5 to 6.0 in a ratio of 1:1, washing twice with 20 mM MES buffer of pH 5.5; adding 0.1 M 1-ethyl-3 (3-dimethyl-amino propyl) carbodiimide hydrochloride (EDC) in 20 mM MES buffer of pH 5.5 to the sonicated latex particles above in a ratio of 1:1, and washing thrice with 20 mM MES buffer (pH 5.5). The latex particles are coated in part by adding 0.6-1.0 mg preferably 0.8 mg per ml of the antibody to the latex particle suspension, stopping the coating reaction by 1M glycine (pH 11.0) taken in quantity of 0.06 ml per ml of solution of antibody coated latex particles, and washing thrice with washing buffer comprised of 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05% sodium azide.

Nilsson discusses a capillary electrophoresis detection system for human chorionic gonadotropin ("hCG") using monoclonal antibodies. There are two different monoclonal antibodies covalently bound to the latex particles. When a test reagent is mixed with hCG containing sample, an immune complex is formed. The complex is separated from the latex particles using capillary electrophoresis and detected by UV-Vis detection system. Since Nillson

discussing using an instrument to detect the hCG, the system is confined to the laboratory, and cannot be used in the field. A capillary electrophoresis is different from an agglutination system.

The present invention is an agglutination test using latex particles coated with gamma-globulin fraction of polyclonal nonspecific serum prepared against S. typhi specific flagellin gene product. Thus, the results can be observed with the naked-eye, and without the aide of any instrument. Consequently, the recited invention can be used in the field.

On page 8-9, the Office Action acknowledges that Nilsson teaches washing with Tris-BSA, and blocking the carboxyl group with Tris-HCl. However, the claim 23 recites that recited invention teaches that the washing step is performed with 20 mM MES buffer of pH 5.5 and that the coating is stopped with 1M glycine (pH 11.0). Since the Office Action does not explain why one of ordinary skill in the art would use MES buffer and 1M glycine instead of Tris-BSA and Tris-HCL, a *prima facie* case of obviousness has not been established.

On page 9, the Office Action acknowledges that Nilsson does not teach preparing an antibody specific to *Salmonella* typhi comprising cloning the Flagellin gene. To address this shortcoming, the Office Action contends that Salzman teaches obtaining flagellin peptides from *Salmonella* species (Office Action at page 9). Salzman is directed to using a monoclonal antibody against a portion/fragment of *Salmonella* muenchen. Salzman discusses using Glutathione-S-transferase (GST) as a tag. Antibodies raised against this protein will not only react with the clinical sample of *Salmonella* muenchen and other gram-negative bacterial infections, but it will also react with parasitic infection caused by *Schistosoma* japonicum. Therefore, specificity of the antibody will be low.

In contrast, the recited invention is directed to using a portion/fragment of flagellin gene sequence specific to S. typhi, and is prepared with polyclonal monospecific antibodies. The gene product comprises 300 amino acids, and does not match with other gram negative bacteria. Therefore, the recited invention is highly specific.

According to the Office Action, Sukosol teaches a recombinant fusion protein of flagellin from S. typhi, and that antibodies against these proteins do not cross react with proteins

from other bacteria (Office Action page 10). However, Sukosol uses a GST as a tag. In contrast, the recited invention is practice using a 6X histine tag containing vector.

Due to the differences discussed above, a combination of the cited references does not result in the recited invention. Additionally, since the references do not teach using a 6X histine tag, the invention is patentable over the cited prior art.

Conclusion

For these reasons, Applicants respectfully request reconsideration and withdrawal of the objections and rejections, allowance of pending claims 23-27, and rejoinder of claim 28.

Respectfully submitted,

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